S-Adenosylmethionine is decreased in the cerebrospinal fluid of patients with Alzheimer's disease

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Abstract: Background: Increased plasma homocysteine levels have been described as an independent risk factor for Alzheimer's disease (AD), but the underlying pathophysiology is unclear. Objective: This single-center, cross-sectional, correlational study analyzed homocysteine metabolism in 60 AD patients and 60 control subjects. Methods: Fasting plasma levels of vitamin B(12), folate and homocysteine as well as cerebrospinal fluid (CSF) levels of folate derivates, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine were measured. In addition, the apolipoprotein E (APOE) genotype was determined. Results: As expected, the APOE4 allele was significantly overrepresented in AD patients compared with controls ($p < 0.001$). Homocysteine plasma levels in the highest quartile were more frequent in the AD patients than in the controls ($p = 0.008$). In addition, AD patients had significantly lower CSF levels of the methyl group donor SAM (193 +/- 31 vs. 207 +/- 37 nmol/l; $p = 0.032$). Accordingly, the SAM/SAH ratio, which represents the methylation capacity, was significantly lower in the CSF of the AD patients (7.6 +/- 2.4 vs. 9.1 +/- 2.8; $p = 0.003$). Further, explorative analysis of all subjects showed that CSF SAM levels were lower in carriers of the APOE4 allele compared with noncarriers (189 +/- 30 vs. 207 +/- 36 nmol/l; $p = 0.010$). Of the individuals with CSF SAM levels in the lowest quartile, 63% carried the APOE4 allele compared with 17% of the individuals with CSF SAM levels in the highest quartile (Pearson: $chi(2) = 9.9$; $p = 0.002$; odds ratio 0.126, 95% confidence interval 0.32-0.49). Conclusion: These data suggest that AD is associated with lower CSF SAM levels and that this is at least partly due to an association of the APOE4 allele with reduced SAM levels in the CSF.

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s-Adenosylmethionine Is Decreased in the Cerebrospinal Fluid of Patients with Alzheimer's Disease

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Key Words
Alzheimer dementia · Homocysteine metabolism · Cerebrospinal fluid

Abstract
Background: Increased plasma homocysteine levels have been described as an independent risk factor for Alzheimer's disease (AD), but the underlying pathophysiology is unclear. ***Objective:*** This single-center, cross-sectional, correlational study analyzed homocysteine metabolism in 60 AD patients and 60 control subjects. ***Methods:*** Fasting plasma levels of vitamin B_{12}, folate and homocysteine as well as cerebrospinal fluid (CSF) levels of folate derivates, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine were measured. In addition, the apolipoprotein E (APOE) genotype was determined. ***Results:*** As expected, the APOE4 allele was significantly overrepresented in AD patients compared with controls ($p < 0.001$). Homocysteine plasma levels in the highest quartile were more frequent in the AD patients than in the controls ($p = 0.008$). In addition, AD patients had significantly lower CSF levels of the methyl group donor SAM (193 ± 31 vs. 207 ± 37 nmol/l; $p = 0.032$). Accordingly, the SAM/SAH ratio, which represents the methylation capacity, was significantly lower in the CSF of the AD patients (7.6 ± 2.4 vs. 9.1 ± 2.8; $p = 0.003$). Further, explorative analysis of all subjects showed that CSF SAM levels were lower in carriers of the APOE4 allele compared with noncarriers (189 ± 30 vs. 207 ± 36 nmol/l; $p = 0.010$). Of the individuals with CSF SAM levels in the lowest quartile, 63% carried the APOE4 allele compared with 17% of the individuals with CSF SAM levels in the highest quartile (Pearson: $\chi^2 = 9.9$; $p = 0.002$; odds ratio 0.126, 95% confidence interval 0.32–0.49). ***Conclusion:*** These data suggest that AD is associated with lower CSF SAM levels and that this is at least partly due to an association of the APOE4 allele with reduced SAM levels in the CSF.

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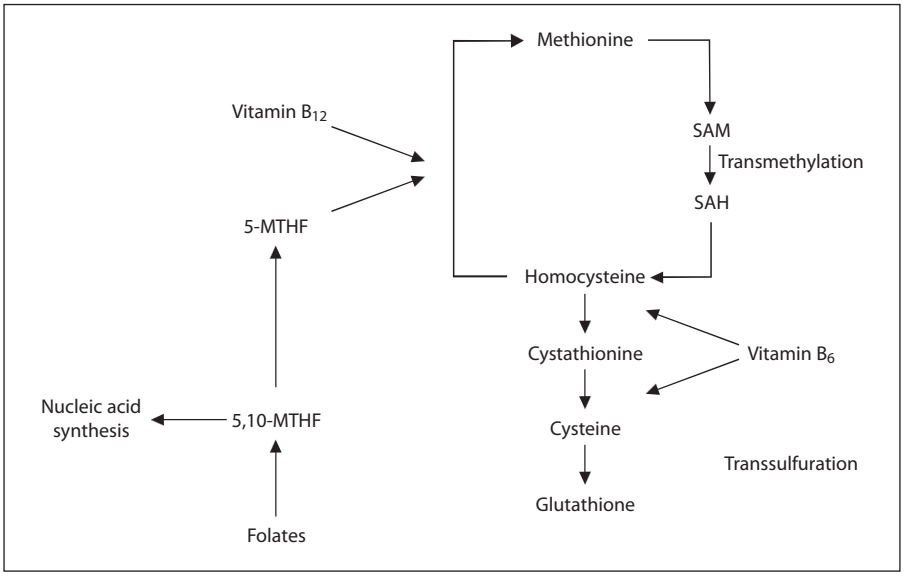

Fig. 1. Homocysteine metabolism. The sulfur-containing amino acid methionine is activated to SAM, which is a ubiquitous methyl group donor. The degradation product of SAM is SAH, which is hydrolyzed to homocysteine. Homocysteine can be remethylated to methionine and SAM, which depends on derivates of folate (5,10-MTHF, 5-MTHF) and vitamin B_{12} as cofactors. Alternatively, homocysteine can be transsulfurated to cysteine as a component of glutathione, which depends on vitamin B_6 as cofactor.

Introduction

Sporadic Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Known risk factors for AD include age, gender, the presence of the apolipoprotein E 4 (APOE4) allele and increased homocysteine plasma levels [1, 2]. Hyperhomocysteinemia, often related to folate or vitamin B_{12} deficiency, is a common finding in the elderly [3] and is associated with cognitive impairment and cognitive decline [4, 5]. The association between hyperhomocysteinemia and AD is well established; however, the underlying pathophysiology remains unexplained. In particular, it is not known whether the homocysteine level itself or other components of the homocysteine metabolism play a role in the development of AD. Recent studies in cell culture experiments and mouse models suggested that 2 metabolites of homocysteine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), may be important in Alzheimer pathogenesis, e.g. by influencing expression of presenilin 1 and β-secretase, leading to an increase in Aβ production [6–8]. SAM serves as a ubiquitous methyl group donor and is necessary, for example, for the synthesis of neurotransmitters, neuronal membrane stability and DNA methylation [9, 10]. After transmethylation, the resulting degradation product of SAM is SAH, which is hydrolyzed to homocysteine (fig. 1).

A few studies have investigated the association between parameters of homocysteine metabolism in the cerebrospinal fluid (CSF) of patients and AD. The comparison of CSF of 8 AD patients and 6 control subjects revealed higher CSF homocysteine levels in the AD group [11]. In contrast, another study investigating CSF of 83 control subjects and 38 AD patients found that homocysteine levels increased with age but were not associated with AD [12]. With regard to SAM and SAH, Mulder et al. [13] investigated the CSF of 30 AD patients and 28 controls and did not observe any significant differences in these parameters between the 2 groups studied. However, this study may have been limited by the fact that 20 of the controls also presented 'subjective memory complaints', but did not have a diagnosis of AD. Another study reported lower CSF SAM levels in 9 patients with AD compared with 29 neurological disease control subjects [14].

In the present study, we compared the fasting CSF levels of SAM, SAH, homocysteine and folate fractions as well as fasting plasma levels of folate, vitamin B_{12} and homocysteine in 60 AD patients with those in 60 controls without cognitive impairment to further investigate the association of the homocysteine metabolism with AD.

Subjects and Methods

The 60 Caucasian study participants with AD were referred to the Memory Clinic, Department of Psychiatry, University of Bonn, for investigation of their cognitive complaints. They met clinical diagnostic criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and Related Disorders Association [15] and DSM-IV criteria for dementia of the Alzheimer type.

Diagnosis of AD was based on neuropsychological and clinical evaluation, including the Mini-Mental State Examination (MMSE), and was approved by a consensus conference of psychiatrists and neuropsychologists prior to the metabolite measurements. The presence of relevant vascular cerebral damage was excluded for all study participants with AD by computed tomography or magnetic resonance tomography and the Hachinsky Ischemic Score (score <4) [16]. Blood and CSF samples from all patients were obtained at the diagnostic workup at which the diagnosis of AD was first made; thus, the age of the patients at the time that the samples were collected was the same as the age at diagnosis.

As disease controls, we recruited 60 consecutive Caucasian patients who underwent lumbar puncture at the Department of Neurology, University of Bonn, for different indications such as exclusion of central nervous system inflammation, exclusion of aneurysmal subarachnoid hemorrhage or exclusion of meningitis. All controls were assessed with the MMSE. Exclusion criteria included age under 18 years, history or evidence of cognitive decline as assessed with the MMSE [17], subjective mental disorders, regular intake of vitamin supplements, neurodegenerative or inflammatory diseases of the central nervous system and other severe or unstable illnesses such as symptomatic cardiac disease, renal or hepatic dysfunction, insulin-dependent diabetes mellitus, untreated thyroidal dysfunction or excessive alcohol intake. In addition, CSF samples indicating blood-CSF barrier disturbances or inflammatory signs, defined as CSF whole protein content >500 mg/dl and more than 5 leukocytes/mm^3, were excluded from both the AD group and controls. Diagnostic lumbar punctures were performed at the Departments of Neurology or Psychiatry, University of Bonn. Lumbar puncture belonged to the diagnostic workup within the clinical routine in all cases of dementia and was not primarily done for study purposes. A standardized technique with a 20-gauge 'atraumatic' spinal needle and the patient in a sitting or lying position was applied. CSF samples were immediately put on dry ice and then stored at –80°C until assayed. Fasting plasma homocysteine concentrations were measured by means of particle-enhanced immunonephelometry with a BN II System (Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting serum vitamin B_{12} and folate concentrations were measured by means of a competitive chemiluminescent immunoassay with an Access™ Immunoassay System (Beckman Coulter, Krefeld, Germany). Deproteinized serum samples for the analysis of SAM and SAH were not available. CSF was analyzed by tandem mass spectrometry for folate derivates [5-methyltetrahydrofolate (5-MTHF), 5,10-methylenetetrahydrofolate (5,10-MTHF), tetrahydrofolate (THF), 5-formyl-THF, folic acid], SAM, SAH and homocysteine as described previously [18, 19]. Homocysteine was measured in CSF by HPLC using fluorescence detection as previously described [20]. Leukocyte genomic DNA was isolated with the Qiagen blood isolation kit (Qiagen, Hilden, Germany). The APOE genotype was determined as previously described [21].

For statistical analysis, univariate analysis of variance (ANOVA) was used to compare CSF and blood parameters between AD patients and controls. CSF SAM levels in APOE4 carriers and APOE4 noncarriers were compared by an independent-samples t test. As age and gender differed significantly between patients and controls, the results obtained from ANOVA analysis were retested by multivariate nominal regression analysis with age and gender as covariables for adjustment of differences. Due to unknown interactions between the blood and the CSF parameters, the multivariate analysis was performed separately for blood and CSF parameters. The association of the APOE4 allele with AD diagnosis was analyzed with Pearson's χ^2 test. Alpha was set as two-sided 0.05. Plasma and CSF levels of homocysteine were compared by Pearson's bivariate analysis of correlation. The study was approved by the local ethics committee. Written informed consent was obtained from all study participants or their trustees.

Results

We compared samples from 60 AD patients (mean age at diagnosis ± standard deviation: 73 ± 8 years; 43 women) with samples from 60 controls (62 ± 10 years; 24 women). The mean MMSE score of controls was 29.0 (95% confidence interval 28.3–29.7) in comparison to 21.5 (20.5–22.5) in the AD patients (ANOVA: $F = 152$; $p < 0.001$). As expected, the APOE4 allele was significantly overrepresented in AD patients compared with controls (87 vs. 27% carriers; Pearson's χ^2 test: $\chi^2 = 38.0$; $p < 0.001$). We did not observe significant differences between AD patients and controls with regard to mean plasma levels of homocysteine, vitamin B_{12}, total folate or creatinine (table 1).

In the CSF, 5-MTHF was present at concentrations higher than the quantification limit in all samples; 5,10-MTHF was detectable in 18 samples, and formyl-THF and THF were detectable in fewer than 10 samples. Folic acid, the synthetic form used for folate supplementations, was not detectable in any of the samples. The quantification limit for the cumulative group of the folate fractions was approximately 0.4 nmol/l. There was no difference in CSF levels of 5-MTHF ($F = 0.54$; $p = 0.464$), 5,10-MTHF ($F = 1.54$; $p = 0.218$), formyl-THF ($F = 0.31$; $p = 0.580$) and THF ($F = 0.05$; $p = 0.818$) between AD patients and controls. Because of the limited number of data, the odds ratio of quartiles was not calculated for CSF folate fractions.

Whereas there was no significant difference in CSF or plasma homocysteine levels between AD patients and controls, the frequency of homocysteine plasma levels in the highest quartile was significantly higher in the AD patients than in the controls ($p = 0.008$; table 1). Accordingly, homocysteine plasma levels in the highest quartile were associated with AD in multivariate nominal regression analysis with age and gender as covariables and with a diagnosis of AD versus control as the dependent variable ($p = 0.023$). AD patients showed significantly lower CSF levels of SAM (ANOVA: $p = 0.032$) and significantly

Table 1. Blood and CSF parameters in AD patients and controls

Parameter	AD patients (n = 60)	Controls (n = 60)	ANOVA	OR quartiles
Homocysteine (plasma), μmol/l	14.1 ± 4.3 (12.9–15.3)	12.7 ± 5.4 (11.3–14.1)	F = 2.20; p = 0.144	4.889 (1.513–15.793); p = 0.008
Vitamin B_{12} (serum), pmol/l	272 ± 192 (219–323)	248 ± 103 (221–276)	F = 0.636; p = 0.427	1.00 (0.350–2.859); p = 0.989
Total folates (serum), nmol/l	15.62 ± 7.04 (13.69–17.52)	14.05 ± 7.74 (11.99–16.09)	F = 1.24; p = 0.269	2.500 (0.828–7.548); p = 0.104
Creatinine (plasma), μmol/l	74.3 ± 13.3 (69.9–77.9)	89.4 ± 77.4 (66.4–111.5)	F = 1.73; p = 0.191	0.650 (0.226–1.866); p = 0.423
SAM (CSF), nmol/l	193 ± 31 (184–201)	207 ± 37 (197–217)	F = 4.74; p = 0.032	0.222 (0.072–0.686); p = 0.009
SAH (CSF), nmol/l	26.9 ± 6.2 (25.2–28.5)	24.3 ± 6.8 (22.5–26.1)	F = 4.44; p = 0.037	3.333 (1.098–10.116); p = 0.034
SAM/SAH (CSF)	7.6 ± 2.4 (6.9–8.3)	9.1 ± 2.8 (8.4–9.9)	F = 9.92; p = 0.003	0.152 (0.048–0.484); p = 0.001
Homocysteine (CSF), nmol/l	71.9 ± 43.5 (59.9–83.9)	77.6 ± 52.6 (63.8–91.4)	F = 0.39; p = 0.536	0.750 (0.262–2.150); p = 0.592

Mean ± standard deviation and 95% confidence intervals (in parentheses) are given. Odds ratio (OR) quartiles: Mantel-Haenszel common odds ratio estimates with asymptomatic 95% confidence intervals (in parentheses) and asymptomatic two-sided significance are given for the distribution of the highest and lowest quartiles of the respective parameter between AD patients and controls. An odds ratio lower than 1.0 means that the lowest quartile was overrepresented in the AD group.

Table 2. CSF levels of SAM (nmol/l) according to the APOE4 genotype

	APOE4 present	APOE4 absent	t test
All subjects (n = 120)	189 ± 30 (n = 50)	207 ± 36 (n = 70)	t = 2.63; p = 0.010
AD patients (n = 60)	188 ± 29 (n = 43)	199 ± 36 (n = 17)	t = 1.10; p = 0.240
Controls (n = 60)	198 ± 34 (n = 7)	210 ± 36 (n = 53)	t = 0.72; p = 0.474

higher CSF levels of SAH (ANOVA: p = 0.037). Accordingly, the SAM/SAH ratio was significantly lower in the AD patients (p = 0.003). When we retested these results in multivariate regression analysis with age and gender as a covariables, the difference in SAM between AD patients and controls was confirmed (Wald = 3.81; p = 0.048), whereas the difference in SAH was not (Wald = 0.022; p = 0.961).

As the APOE genotype is a major risk factor for AD, we next tested whether the observed associations between metabolites and AD were independent of the APOE genotype. When the APOE genotype was added as a covariable to the multivariate model, regression analysis showed no association between AD and SAM levels (Wald = 0.460; p = 0.543). Thus, we suspected that CSF levels of SAM were associated with the APOE genotype, and we additionally tested metabolite levels in strata of the APOE genotype. While the other parameters showed no significant differences, CSF SAM levels were significantly lower in the carriers of the APOE4 allele (ANOVA: F = 6.93, p = 0.010, for AD patients plus controls). This association was significant when patients and controls were analyzed together (table 2), but not when patients and controls were analyzed separately. Of the individuals with CSF SAM levels in the lowest quartile, 63% carried the APOE4 allele compared with 17% of the individuals with CSF SAM levels in the highest quartile (Pearson: χ^2 = 9.9; p = 0.002; odds ratio 0.126, 95% confidence interval 0.32–0.49).

In a generalized linear model, there was no significant interaction between AD diagnosis and the APOE4 allele with regard to SAM levels (not shown). SAM levels did not correlate with the MMSE score in the AD patients.

Discussion

In the present study, several parameters of homocysteine metabolism were analyzed in the CSF and plasma of 60 AD patients and 60 control subjects without cognitive impairment. Due to differences in age and gender between patients and controls as a limitation, we performed multivariate analysis with age and gender as covariables in addition to univariate analyses. Whereas homocysteine plasma levels were not significantly associated with AD in ANOVA, individuals with homocysteine

plasma levels in the highest quartile significantly more often belonged to the AD group in comparison to individuals with homocysteine levels in the lowest quartile. This is in line with the known association between elevated homocysteine plasma levels and AD [1]. However, we did not observe higher homocysteine CSF levels in AD patients as previously reported [22].

In addition, we did not detect significant differences in blood levels or quartiles of vitamin B_{12}, folate or creatinine, nor in CSF levels or quartiles of folate fractions. However, in the AD patients, we observed significantly lower CSF levels of SAM and a significantly lower SAM/SAH ratio. As the observed lower levels of SAM in CSF were also significant in a multivariate model, this difference is unlikely to be explained by differences in age and gender between AD patients and controls. This confirms a previous report by Bottiglieri et al. [14], who observed lower CSF levels of SAM in 9 AD patients in comparison to 29 patients with other neurological diseases. Accordingly, in a postmortem analysis, the concentration of SAM was severely decreased in the brains of AD patients [23].

Due to the role of SAM as a ubiquitous methyl group donor and SAH as a strong inhibitor of SAM-dependent transmethylation reactions, lower levels of SAM and higher levels of SAH are supposed to result in a reduced methylation capacity [24]. It was previously suggested that low SAM levels are associated with DNA demethylation followed by increased expression of presenilin 1 and β-secretase, leading to an increase in Aβ production. Supplementation of SAM prevented these changes in cell culture experiments and mouse models [6–8]. Additionally, high concentrations of the hyperphosphorylated tau protein in CSF, which predict the development of dementia [25], were associated with low activity of the SAM-dependent protein phosphatase PP2A [26]. Thus, low brain or CSF levels of SAM and high levels of the SAM inhibitor SAH might promote both Aβ production and accumulation of hyperphosphorylated tau protein. Another aspect of a possible association between methionine metabolism and neurodegenerative disorders is oxidative stress. The transsulfuration reaction of homocysteine to cysteine, a precursor of glutathione, is activated by SAM [9]. Therefore, a lack of SAM may result in a reduced antioxidative capacity and increased oxidative stress, a hallmark of several neurodegenerative conditions including AD [27, 28]. Recent findings showed that oral substitution of SAM results in an increase in both plasma and CSF levels of SAM, and SAM substitution may effect some clinical improvement in AD patients [29].

The second interesting observation in our study was the association of CSF levels of SAM with the APOE4 allele. Knowing that these results are limited by the small sizes of the respective subgroups and by the limitations of pooling patients and controls for such analyses, the association of SAM levels with the APOE4 allele was not significant in separate analyses of patients and controls, although both (as yet) healthy and demented carriers of the APOE4 allele had lower levels of SAM than noncarriers. This observation leads to the question whether there is a biological effect of APOE on SAM, which remains speculative.

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